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TO: See Distribution List

FROM: Martha Lamont, Director
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SUBJECT: Microbiological Data Program Plan, July through December 2006

This Program Plan serves as the current Statement of Work for the period July 1, 2006, through December 31, 2006, for each State participating in the Microbiological Data Program (MDP). This document also stipulates work assignments for the Federal facility participating in MDP.

I. ADMINISTRATIVE UPDATES

A. Personnel: Program participants are reminded to keep MDP management informed of any critical equipment purchases, staffing issues, or expected increases in rent or sample turn-around-time (e.g., due to laboratory or office renovation/relocation). This information is required under the terms of MDP Cooperative Agreements (Section II, Responsibilities) between USDA and participating States.

Dr. Jane Tang, MPO Microbiologist, resigned effective April 25, 2006. Donna Dickriede, MPO Microbiologist, resigned effective May 12, 2006. Modifications to laboratory liaison assignments were communicated to MDP laboratories.

B. Summary Status: The 2004 and 2005 MDP Data Summaries have been released and over 250 hardcopies were shipped to various individuals and agencies. The 2004 and 2005 MDP Data Summaries are also available on the MDP website at http://www.ams.usda.gov/science/MPO/MDPSumm05.pdf.

C. Financial/Cooperative Agreements: Cooperative agreements for Fiscal Year (FY) 2006 have been issued. Beginning April 1, 2006, the California Department of Food and Agriculture laboratory (CA4) was no longer an MDP participant. Samples collected by California are analyzed by the Ohio laboratory (OH4) and the AMS National Science Laboratory [NSL (US4)].

D. MDP Program Meetings: A Technical/Quality Assurance (QA) Meeting was held March 29-31, 2006, at the Virginia Division of Consolidated Laboratory Services (DCLS), Richmond, Virginia. Technical and QA issues were addressed in addition to a QA session that focused on ISO accreditation.

On July 16-19, 2006, the Florida Department of Agriculture and Consumer Services will host the 8th Annual Foodborne Pathogen Analysis Conference (FPAC). The purpose of this meeting is to present technological/methodological updates and to present and discuss issues regarding food safety. In conjunction with the conference, MDP will meet to discuss planning and technical issues currently facing the program.

E. Electronic Transfer of Data:

RDE Version Upgrades: The current version of the Web-based RDE system was installed in June 2005. The next version upgrade is tentatively scheduled for July 2006. The report generation problems will be addressed in the next version upgrade. A version upgrade of the RDE electronic Sample Information Form (e-SIF) system for laptops/palmtops was distributed in December 2005 to add selection lists for populating the post-harvest treatment field and to allow the user to change an existing Sample ID that has an incorrect entry. MPO maintains a Change Request Database to capture all problems identified and suggestions made regarding the RDE system.

E-SIF History Database Maintained at MPO: A new database has been implemented at MPO to capture each e-SIF record submitted by sample collectors. The e-SIF clearinghouse system at MPO uses this database to identify and block duplicate e-SIFs. The database will be used by the MPO Sampling staff to analyze trends and ensure that required information is being entered on the SIFs. The database allows MPO to generate a summary report of State e-SIF counts, including the number of reported samples versus the actual number expected. The database should allow MPO to respond quicker to laboratory inquiries about missing e-SIF records.

II. PROGRAM SAMPLING AND TESTING UPDATES

A. Sampling: Shipping Charts are distributed quarterly to Sampling Managers by MPO. Cantaloupe, tomatoes, green onions, lettuce (leaf or romaine), and alfalfa sprouts will continue. Samples collected in Maryland will be sent to the Ohio laboratory (OH4) and those collected in Texas will be shipped to NSL (US4). Cantaloupe samples collected in California will be sent to NSL (US4); all other commodity samples collected in California will be sent to the Ohio laboratory (OH4). Samples from all other States will be sent to the laboratory for that collection State. Beginning July 1, 2006, Roma tomatoes will be an acceptable tomato variety. Up to three Roma tomatoes per laboratory sample may be collected to satisfy sample weight requirements.

B. Testing: Cantaloupe, tomatoes, green onions, lettuce (leaf or romaine), and alfalfa sprouts will continue. The Ohio laboratory (OH4) will analyze all samples collected by Ohio and Maryland as well as tomatoes, green onions, lettuce, and alfalfa sprouts collected by California. NSL (US4) will analyze all samples collected by Texas and cantaloupe samples collected by California. All other States will analyze samples collected in that State.

Target Microorganisms:

(1) *Escherichia coli* (*E. coli*): MDP laboratories will continue testing all samples for *E. coli* using ColiComplete®. Method procedures are detailed in Standard Operating Procedure (SOP) MDP-MTH-01, *Escherichia coli* MPN Method. A 10% decline in the number of MUG (*E. coli*)-positives has been observed since the January 2006 introduction of Universal Preenrichment Broth (UPB) as the wash and enrichment broth. This observation and associated issues, including the addition of Tween to the wash and a possible return to Buffered Peptone Water (BPW) + 0.1% Tween (for the wash step only), were discussed during the March Technical/QA Meeting.

(2) Pathogenic *E. coli*: MDP laboratories will screen samples that were positive for *E. coli* (by SOP MDP-MTH-01) for pathogenic *E. coli* according to SOP MDP-MTH-07, Detection of Pathogenic *E. coli* in Fresh Produce by Multiplex PCR (mPCR) and Cultural Isolation and Identification. The mPCR assay tests for two types of pathogenic *E. coli*: (a) shiga toxin-producing *E. coli* (STEC) which contain genes coding for shiga toxins (Stx-1 and Stx-2) and (b) enterotoxigenic *E. coli* (ETEC) containing genes coding for the heat labile (LT-1) and heat stable (ST-1) toxins. Cultural procedures for the isolation of pathogenic *E. coli* are included in SOP MDP-MTH-07.

(3) *Salmonella:* MDP laboratories will continue to screen all samples for *Salmonella* (presence or absence) by BAX®. Method procedures are detailed in SOP MDP-MTH-04, Detection of *Salmonella* in Fresh Produce by BAX® PCR. Presumptive positive samples are subjected to enrichment and isolation as described in SOP MDP-MTH-03A, Isolation and Identification of *Salmonella* from Fresh Produce Using Cultural Methods.

(4) *E. coli* O157:H7: MDP laboratories will continue to screen all samples for *E. coli* O157:H7 (presence or absence) by BAX®. Method procedures are detailed in SOP MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX® PCR. Presumptive positive samples are subjected to immunomagnetic separation (IMS) procedures and cultural confirmation, as described in SOP MDP-MTH-06, Isolation and Identification of *Escherichia coli* O157 by Immunomagnetic Separation (IMS) and Cultural Methods.

C. Quality Assurance:

Proficiency Testing Program: The next proficiency testing (PT) round, which will include determination of *E. coli* O157:H7 using a non-toxigenic strain, is currently under development. Future PT plans will include the requirement of only one round per year. Future rounds may contain multiple target organisms to be identified and reported by participating laboratories using program SOPs. All PT rounds are announced in the MDP Semi-Annual Program Plans.

Method Verification Report: The final report on Method Verification for Alfalfa Sprouts, UPB, and DNA Extraction performed by each participant was completed and sent to program participants on January 27, 2006.

SOPs: SOPs are posted to the MDP website when distributed to program participants. http://www.ams.usda.gov/science/MPO/SOPs.htm.

The following SOP was distributed March 1, 2006:

- MDP-QA-02, Proficiency Test Samples, Revision (Rev) 01

The following SOPs were distributed May 1, 2006:

- MDP-LABOP-02, Sample Receipt, Elution, Preenrichment, and DNA Extraction, Rev 06
  Attachment 01, Fabrication of the California Cantaloupe Shaker Adapter
  Attachment 02, Sample Receipt Form (SRF) – downloadable version available on website at http://www.ams.usda.gov/science/MPO/Mdp.htm
- MDP-LABOP-03, Microbiological Media & Reagents, Rev 02
- MDP-LABOP-08, Procedure for Testing and Maintaining Control Strains, Rev 03
- MDP-DATA-01, Record Keeping and Results Reporting, Rev 03
  Attachment 01, Preliminary and Final Results Notification Form
- MDP-DATA-02, Data Storage and Archival, Rev 01
  Attachment 01, MDP Designated Federal Records Centers
  Attachment 02, Standard Form (SF)-135 (template plus example)
  Attachment 03, Instructions for Assembly and Packaging of Record Boxes
- MDP-QA-01, Laboratory Equipment Preventive Maintenance, Rev 02
- MDP-ADMIN-01, Facilities, Rev 01
- MDP-ADMIN-02, Personnel and Organization, Rev 01
- MDP-ADMIN-03, Training/Personnel Records, Rev 01
- MDP-ADMIN-04, Laboratory Equipment, Rev 01
- MDP-ADMIN-05, Safety, Rev 01
- MDP-ADMIN-06A, USDA/AMS Quality Assurance Unit (QAU), Rev 01
- MDP-ADMIN-06B, Laboratory Quality Assurance Unit (QAU), Rev 01
- MDP-ADMIN-07, Preparation and Maintenance of Standard Operating Procedures (SOPs), Rev 01

D. Archiving and Additional Testing:

Archival of Isolates: NSL (US4) serves as a centralized location for archival of isolates as well as a distribution center for isolates from MDP testing laboratories to the reference laboratories.

Additional Testing by Reference Laboratories: All target organisms are frozen in Microbank™ vials and shipped to NSL (US4). Vials are shipped by NSL (US4) to the FDA/Center for Veterinary Medicine (CVM) laboratory in Laurel, Maryland for antimicrobial resistance testing for inclusion in the National Antimicrobial Resistance Monitoring System (NARMS) and pulsed-field gel electrophoresis (PFGE) analysis for inclusion in PulseNet. *Salmonella* and *E. coli* O157 isolates are also serotyped by FDA/CVM. Pathogenic *E. coli* isolates are shipped by NSL (US4) to Pennsylvania State University for serotyping.

E. Transfer of Data: AMS transfers data to the Centers for Disease Control and Prevention (CDC) and FDA on a semi-annual basis.

F. Future Program Directions:

Shigella: MPO contracted the development of a realtime PCR-based method for the detection of *Shigella* spp. in produce to DCLS. Microbiologists at DCLS tested various primer and probe sets for a TaqMan® PCR method as well as several primer sets derived from the 16S rDNA sequence for use as an internal control. This method development also included cultural media for the differentiation and isolation of *Shigella* from generic *E. coli* and other background bacteria. DCLS demonstrated their method during the March Technical/QA meeting and will provide a method try-out protocol for MDP participating laboratories to use in May 2006.

FlashGel™: FlashGel™ offers a rapid method for separating DNA fragments and visualizing them during migration through a precast agarose gel. The system does not require ethidium bromide staining or illumination with ultraviolet (UV) light. The entire process can be completed in less than 10 minutes and will help accelerate isolation of target pathogens from PCR-positive cultures. The Ohio laboratory (OH4) tested the FlashGel system and presented results at the March Technical/QA meeting. Laboratories are encouraged to pursue the use of FlashGel™ by requesting a deviation from MPO; an authorization letter detailing the verification protocol will then be issued.

Collaboration with FDA: MDP participating laboratories in Ohio (OH4), Florida (FL4), New York (NY4), and NSL (US4) will each ship 50 leftover produce washes to Dr. Palmer Orlandi at the FDA/Office of Applied Research and Safety Assessment in Laurel, Maryland. The washes, obtained from all current MDP commodities and sampled from California, Florida, Maryland, New York, Ohio, and Texas, will be analyzed for the presence of *Cyclospora cayetanensis* and *Cryptosporidium parvum*. These waterborne coccidian parasites are considered emerging human pathogens; the general understanding of their epidemiology is limited. Dr. Orlandi and his group have published several papers on these organisms and their PCR-based protocols are posted on FDA/CFSAN and FERN websites.